CONTROL OF FLASHING IN FIREFLIES. I. THE LANTERN AS A NEUROEFFECTOR ORGAN

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The ability of some fireflies to produce remarkably uniform and brief flashes of light is of much interest in relation to cellular control mechanisms. Neural involvement has been implicit or explicit in most theories of flash regulation, but there are two main ideas as to its ultimate mechanism. According to one view the luminescence of the photocyte is controlled by limiting the access of oxygen by supposed mechanical valves (tracheal end cells). According to the other view the terminal nerves stimulate the photocyte directly. The oxygen theory has been dealt with in previous papers (Buck, 1948; Hastings and Buck, 1956). The present series of papers explores responses to electrical stimulation.

Work by Macartney (1810), Macaire (1821), Todd (1826), Joseph (1854), Kölliker (1858), Owsjannikow (1868), Bellesme (1880), Arnold (1881), Verworn (1892), Perkins (1931), Brown and King (1931), Snell (1932), Alexander (1943) and others showed that voluntary luminescence is abolished by decapitation and can be elicited by electrical stimulation. Dubois (1886), Heinemann (1886), Fuchs (1891), Lund (1911) and Gerretsen (1922) attempted to localize the stimulation to specific efferent nerves, though at a very crude level. Steinach (1908) reported evidence of summation of subliminal stimuli, and Chang (1956) recorded facilitated series of responses from both the larval and adult firefly and compared them with responses in muscle. Hanson (1961) showed that regional excitation corresponds to the gross innervation of the lantern, and Carlson (1961) found that even the "pseudoflash," caused by passive entry of oxygen into an hypoxic lantern, is affected by prior neural stimulation. Nonetheless, the type and degree of neural involvement in flash control remain uncertain because of ignorance of where the nerves in the lantern terminate. Thus, nerves might control either end cells (oxygen control) or photocytes directly, and the hypoxic effect might be a direct limitation or, as Brücke (1881) suggested long ago, an inactivation of control nerves.

In view of this impasse it seemed possible that understanding of the role of nerve in triggering the firefly flash might be gained by analyzing the flash as an effector event and exploring its variation with changing stimulus parameters. Subsequent papers in this series will deal with central nervous aspects of excitation, peripheral nervous phenomena, and effector unit response. Preliminary summaries of some of the work have been given (Case and Buck, 1957, 1958, 1959a, 1959b).

[1] Supported by PHS research grant B 1890 from the National Institute of Neurological Diseases and Blindness.

Materials and Methods

The fireflies investigated were adults of *Photinus pyralis* (Linné) from Maryland and Iowa, *Photinus marginellus* Le Conte and *Photinus consanguineus* Le Conte from Woods Hole, *Photinus punctulatus* Le Conte from Iowa, and both larvae and adults of the most common photurid (tentatively identified as *Photuris versicolor* Barber) from each of the three localities. (Identifications were made via the papers of Barber and McDermott (1951) and Green (1956).) Adult specimens were netted in the field and used within a week, meanwhile being kept unfed in humidified jars at room temperature. Larvae were collected on lawns near streams and kept in petri dishes on moist paper, either at room temperature for immediate use or at 5–10° C. for use weeks or months later.

Preparations included intact individuals, decapitated specimens, and isolated lanterns. To prepare the adult lantern, which consists of ventral plaques in abdominal segments 6 and 7, these segments were excised and all viscera overlying the photogenic organ, usually including the terminal ganglia of the ventral nerve cord, were removed. The larval light organs, a pair of small oval spots on the ventrolateral surface of abdominal sternite 8, were excised with part of the surrounding sternite.

Stimuli were delivered by Grass S-4 stimulators via radio-frequency isolation units and electrodes of unshielded 30-gauge silver or platinum-iridium wire. Intact or decapitated specimens were de-legged and anchored on dental wax with wire staples spanning the body transversely. Isolated light organs were laid across a parallel electrode pair in a small moist chamber.

Two methods were used in temperature experiments. In one, the specimen, with electrodes in position, was bound to the bulb of a thermometer reading to 0.01° suspended in air in a test tube immersed in a water bath; in the other the specimen was attached to a thermistor thermometer readable to 0.5°, in a small copper air chamber through the walls of which water was circulated from a large temperature-stabilized reservoir.

Light emission was followed with an RCA 931-A photomultiplier leading directly to one channel of a dual beam oscilloscope and photographed simultaneously with a second trace carrying a stimulus marker.

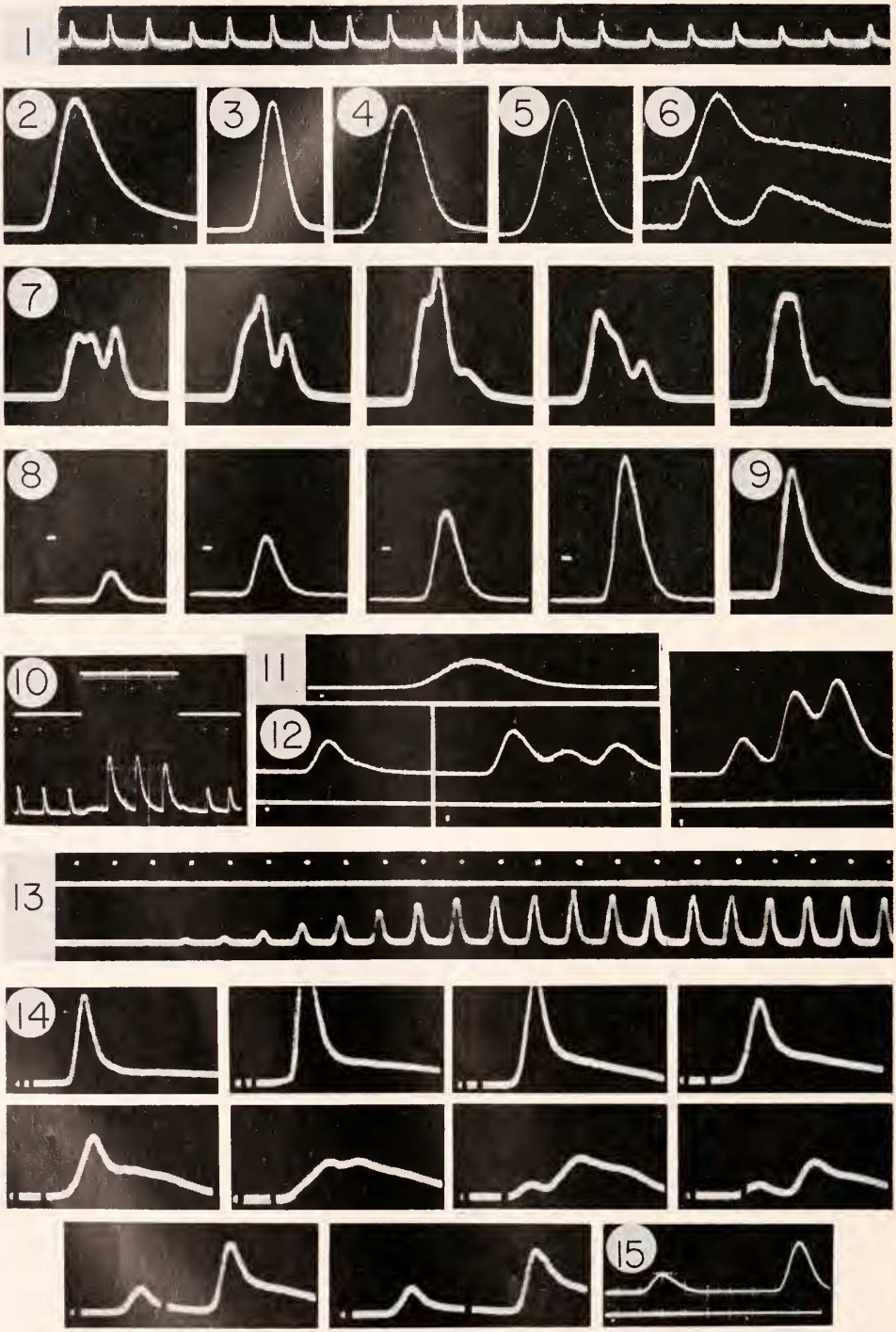
In all except the temperature experiments the preparation was mounted on the stage of a stereoscopic microscope magnifying up to 120×. Unless otherwise specified, all experiments were done at 25 ± 3° C.

Results

Both spontaneous and induced flashing exhibit a high degree of consistency, and most of the illustrations and data presented are typical of responses obtained in dozens or hundreds of measurements. Instances of refractoriness associated with apparent central nervous phenomena will be considered in the following paper.

1. *The spontaneous flash*

Spontaneous flashing is seen only in individuals with brain still connected with cord. As a general rule it does not occur in either field or laboratory below 18–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20° C. Under the conditions of most of our experiments (animal immobilized; electrodes inserted), spontaneous flashes were generally infrequent, although single responses could sometimes be induced by various types of mechanical stimulation. *Photuris* was exceptional in its spontaneous activity, some specimens flashing with almost clock-like regularity. Figure 1 shows the first 10 and last 10 of 80 consecutive flashes given by a male of *Photuris* in 32 seconds. In this series the mean interflash interval was 405 ± 3.2 milliseconds (S.E. = 0.8% of mean) and mean flash intensity was 21.3 ± 0.5 arbitrary light units (S.E. = 2.4% of mean).

Insofar as flash kinetics are concerned, oscilloscopic observation confirms field impressions in showing characteristic differences between species. Thus, the durations of flashes of comparable magnitude may vary from 200 msecs. or less in *Photuris* (Fig. 9) to a second or more in *Photinus pyralis* (Fig. 2). Single flashes may have nearly symmetrical accretion and decay phases (Figs. 3, 4, 5) or show marked skewing (Figs. 2, 9). Flashes may be either single or multiple (Figs. 6, 7). We have already emphasized the usual constancy of flash type within a species (Figs. 1, 13, 32 etc.) but it is also relevant to the control problem to point out differences that may occur between the flashes of the two sexes of one species (Figs. 6 *vs.* 3 *vs.* 9), between different individuals of the same sex and species (Figs. 3 *vs.* 9; 6), and even between successive flashes of one individual (Fig. 7). Sometimes these variants have ready explanations, such as differences in degree of disturbance of the specimens, or asynchrony or variable involvements of different lantern regions; but some are not presently understood.

In most species the spontaneous flash under laboratory conditions appears comparable to that emitted in natural flight. However, in *Photuris*, which is a variable and confusing genus even in field behavior (Barber and McDermott, 1951), the free-flying male of the Woods Hole variety typically gives a rapid four-peaked twinkle every second or two, whereas the female gives a long lingering glow at irregular intervals; in the laboratory both sexes usually give single flashes, although

FIGURES 1–15. Note that in photos of oscilloscope traces the time scale (S) is given as the width of the print. Some stimulus artifacts have been retouched. (1) Woods Hole *Photuris*, male. First and last 10 of a series of 80 spontaneous flashes. S = 8 sec. (2) Maryland *Photinus pyralis*, male. Spontaneous flash. Light did not regain base level for 1 second. S = 735 msecs. (3) Woods Hole *Photuris*, male. Spontaneous flash. S = 220 msecs. (4) *Photinus marginellus*, male. Spontaneous flash. S = 450 msecs. (5) Maryland *Photuris*, male. Spontaneous flash. S = 130 msecs. (6) Woods Hole *Photuris*, female. Two frames of spontaneous flash of two individuals. S = 300 msecs. (7) *Photinus consanguineus*, male. Five successive spontaneous flashes. S = 640 msecs. (8) Woods Hole *Photuris*, male. Responses of isolated lantern to 5, 7, 8 and 9 volts, all at 10 msecs. S = 200 msecs. (9) Woods Hole *Photuris*, male. Spontaneous flash. S = 240 msecs. (10) Woods Hole *Photuris*, female, decapitated, electrodes in light organ. Eight successive responses to 10 msecs./15 v. with the fourth, fifth and sixth occurring in a 4 v. D.C. field. S = 10.5 secs. (11) *Photuris* larva, decapitated, electrodes immediately anterior and posterior to light organ. Induced glow, 10 msecs./15 v. S = 3.6 secs. (12) *Photinus punctulatus*, female, isolated lantern. Three frames showing responses to 4, 8 and 10 v./4 msecs. S = 350, 500, 500 msecs. (13) Woods Hole *Photuris*, sex unknown, decapitated, electrodes in lantern. Response to stimulus train of 4 msecs./5 v. at 10 per second. S = 2.2 secs. (14) Woods Hole *Photuris*, female, decapitated, electrodes in lantern. Responses to paired stimuli of 6 msecs./15 v. with intervals of 15, 20, 30, 40, 50, 60, 70, 90, 150 and 200 msecs. S = 360 msecs. except 430 msecs. for last two frames. Stimulus indicated by break in photomultiplier trace. (15) Maryland *Photinus pyralis*, male, decapitated, electrodes immediately anterior and posterior to lantern. Responses to two equal stimuli 1200 msecs. apart. S = 2 secs.

at least the female occasionally produces compound flashes (Fig. 6) as does the female of the Maryland *Photuris* (Fig. 8 of Hastings and Buck).

In larvae of most lampyrid fireflies the photogenic organ is much smaller and simpler than the adult lantern, and light is emitted as a long glow rather than a short flash. No spontaneous glows were recorded, because of their very irregular timing and low frequency, but the kinetic contrast between larval and adult luminescence is well illustrated by the induced glow (Fig. 11; note time scale). Actually, the duration of the larval glow is usually even longer in nature—often several seconds—but in view of our observations at different temperatures (see below) this is quite possibly accounted for by the temperature of the soil surface being considerably below 25°, particularly on the damp evenings in early autumn when larvae are most easily observed.

2. *Induced flashing : general*

Although, as will be shown, it is possible to alter flash duration, intensity and form by changing stimulus parameters, a single, moderate electric shock usually elicits a flash indistinguishable from the normal spontaneous flash characteristic of the species. Seemingly normal flashes can result with the electrode pair at any level of the body from head to lantern, but are usually most reliably produced with ab-

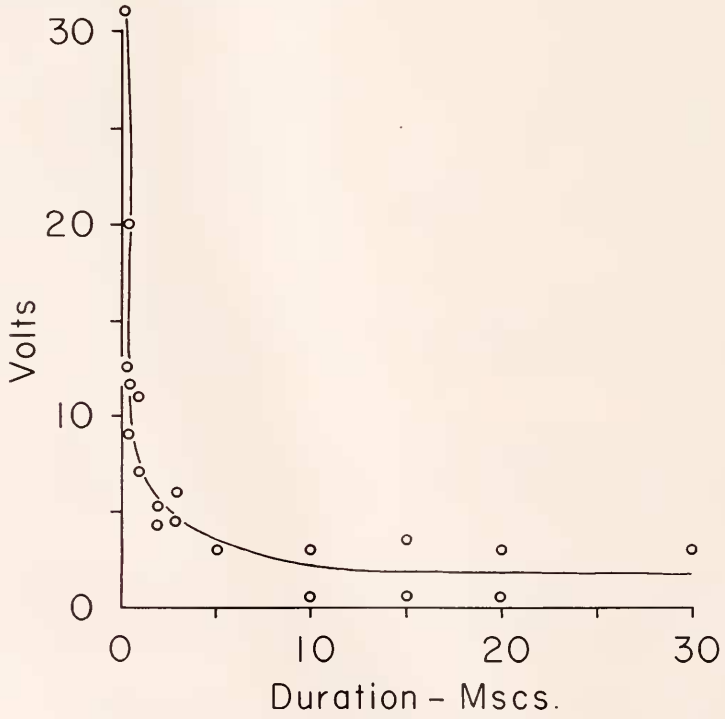


FIGURE 16. Representative strength-duration curve for minimum visually detectable response induced by direct stimulation of lantern of a decapitated Woods Hole *Photuris* male.

dominal stimulation. In the observations described below, induced responses, unless specifically excepted, were recorded with electrodes either in abdominal segment 6 at the level of the last ganglionic mass of the cord, or actually inserted in lantern tissue. In both instances excitation is presumably *via* peripheral nerve.

In connection with changes in flash characteristics, it is important to avoid being misled by differences due simply to magnitude. In Figure 39, for example, the weakest and strongest flashes might be thought to be quite different in form, whereas in fact the relative rates of rise and fall in light intensity are the same in both, and the two flashes could be made to coincide by equalizing their peaks.

3. *Threshold*

The isolated light organ, 15 minutes after preparation, gives reproducible threshold values apparently not much affected by unavoidable variations in tissue-electrode contact. Using this preparation, the interrelations of stimulus voltage and duration for minimum detectible response were studied. The results (Fig. 16) show strength-duration relations similar to insect nerve-muscle. In seven males of *Photuris* with electrodes in the lantern the average rheobase was 2.1 volts and the chronaxie was 3.9 mscs. Minimum values were 0.7 volts and 1.7 mscs., respectively. Values for the female of *Photuris* and for males of *Photinus pyralis* and *P. punctulatus* were in the same range as for the male of *Photuris*.

Threshold for direct current stimulation is relatively uniform. In *Photinus pyralis*, for example, the values in three individuals were 5.5–6.0, 3.5–4.0 and 7.5–8.0 volts.

Threshold is much increased at lower temperatures. In typical measurements on *Photuris* a 10 mscs./6 v. shock elicited a flash of good intensity at 25°, whereas, in the same individual, 50–60 volts at the same duration were required in the 9–12° range to obtain even a much weaker response.

4. *Factors affecting flash intensity*

a. *Stimulus strength and duration.* Within limits, flash intensity varies with voltage of single shocks of given duration (Figs. 8, 17). The same effect is seen during stimulation imposed upon D.C. fields (Fig. 10). In certain species, particularly if the lantern still has central connections, increasing strength of stimulus may induce multiple flashes (Fig. 12). These probably autoexcitatory responses will be considered in detail in the second paper of this series.

As would be expected from the strength-duration findings, variation in stimulus duration alone also affects flash intensity. These effects are considered in the section on flash form.

b. *Effects of stimulation frequency on flash intensity: facilitation.* With subliminal stimulation, the adult firefly lantern exhibits typical summation, response first occurring after several shocks and then growing (Figs. 13, 44). Additive effects occurring with supra-threshold stimuli are well displayed by paired shocks (Figs. 14, 18). The minimum delay which produced detectible summation (*i.e.*, the absolute refractory period) was not investigated in detail, but is clearly less than 5 mscs. in *Photuris* and is apparently between 6 and 8 mscs. in *Photinus pyralis*, using near-threshold stimuli in both instances. Above these minima, facilitation

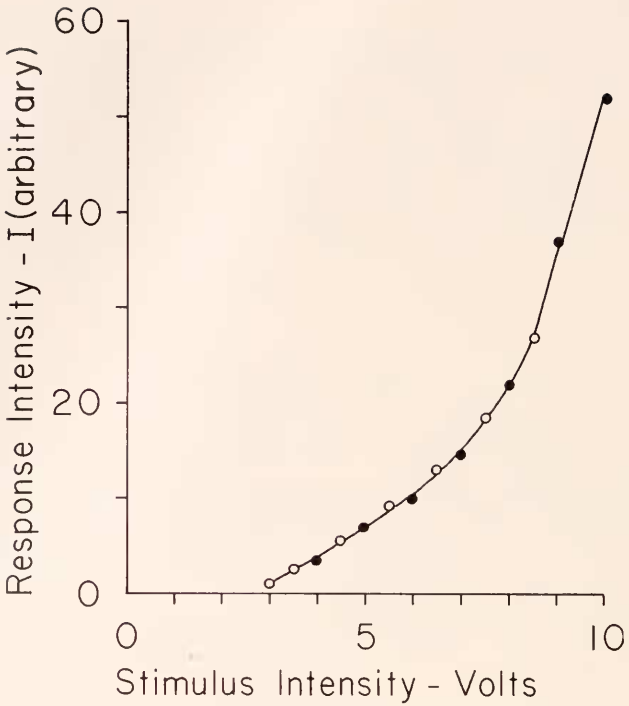


FIGURE 17. Relationship between stimulus strength and flash intensity at constant 10 mscs. stimulus duration. Open circles represent ascending voltage series, closed represent descending series. Woods Hole *Photuris* male, isolated lantern.

increases rapidly to a maximum that shows considerable variation both intra- and inter-specifically. In six specimens of *Photuris* maximum facilitation occurred with a stimulus interval of 10–20 mscs. (Fig. 18), but in two other individuals the most effective intershock interval seemed to be at least 30 mscs. In most specimens of *Photinus pyralis* the buildup and decay of facilitation was more gradual, the most effective delay lying between 50 and 100 mscs.

After maximum facilitation is attained, flash intensity declines sharply with increasing delay. In *Photuris* the facilitating effect of the second shock is practically dissipated by the time the delay reaches 60 mscs.

Concurrently with changes in flash intensity, increasing intershock delay causes changes in flash form, manifested by a slowed rate of rise without change in latency —*i.e.*, a shift in peak position toward later time (Fig. 14, first *vs.* fifth frames), relegation of the original flash to a shoulder on the rise phase of the second response (Fig. 14, sixth and seventh frames), and finally by a splitting of the flash into two peaks representing the separate responses to the two shocks (Fig. 14, eighth and ninth frames). In *Photuris* the separation is complete at an inter-stimulus interval of approximately 85 mscs: in *Photinus pyralis* not until 300 mscs.

At the interval for which separate responses to paired shocks are first detectible, the first flash is sometimes the more intense. However, as the peaks draw apart

the second usually becomes larger than the first (Fig. 14, frame seven *et seq.*). In both *Photuris* and *Photinus pyralis* this apparent facilitation of one response by the preceding one persists, in some instances, for more than a second (Fig. 15).

Facilitation is also shown by the *Photuris* larva, according to data collected by Dr. Albert Carlson (Fig. 19). Here the maximal summation effect of the second stimulus on the response to the first is reached only after 50–60 mscs., and persists at least 500 mscs., a much longer time than in the adult. The augmentation of the second response of a pair as a function of the first response was not investigated in larvae, but, if comparable to other adult/larval response rate ratios, would be expected to persist at least 10 seconds.

When repetitive stimulation is extended from paired shocks to trains, response varies widely depending on stimulus frequency and voltage, temperature, degree of neural integrity, degree of facilitation, and species. Figures 13 and 22 illustrate the rather stable sort of response seen typically with moderate stimulation, in which each flash is independent and the responses increase progressively in intensity and duration for several successive stimulation intervals, then reaching a plateau. As might be expected, the gain in flash intensity is usually more rapid the higher the rate of stimulation (Figs. 20, 21), and in species or individuals producing double flashes, both flash components augment (Fig. 22).

The stimulation frequency limit for production of completely or partially independent responses of course varies with flash duration, among other factors. In

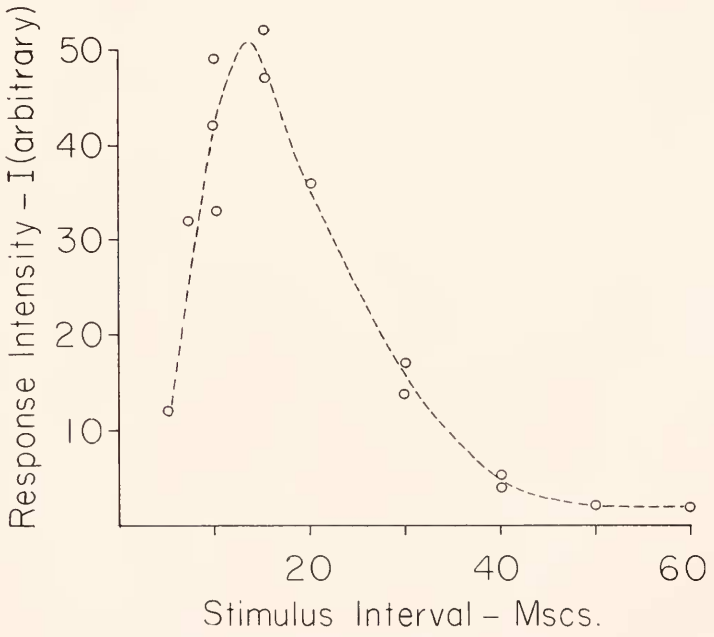


FIGURE 18. Temporal facilitation of electrically induced flashes. Abscissa represents interval from first to second stimulus of a pair, in all instances 3 mscs./5 v. Woods Hole *Photuris* female, isolated lantern. Typical of five other specimens.

Photuris, completely separate responses to each stimulus of a train can usually be elicited at frequencies of at least 10/sec. (Fig. 13), whereas in *Photinus pyralis* any frequency higher than about one per second prevents the luminescence from falling to baseline before the next response. As stimulation frequency is still further increased the individual flashes necessarily begin to merge, the fusion giving a maintained luminescence that changes more or less in proportion to flash intensity, and, in combination with facilitation, may produce responses quite similar to the familiar staircase and tetany of striated muscle (Figs. 23, 24). However, it is not uncommon, when close to the limit of 1 : 1 response to continued serial stimulation, to find the average response intensity falling off markedly after a brief rise (Figs. 25, 27, 30, 33).

Species differences are strikingly illustrated in ability to follow the stimulation frequency. The Woods Hole photurid, for example, is able to respond 1 : 1 up to about 20 shocks per second (Fig. 27). The Iowa photurid fails at between 10 and

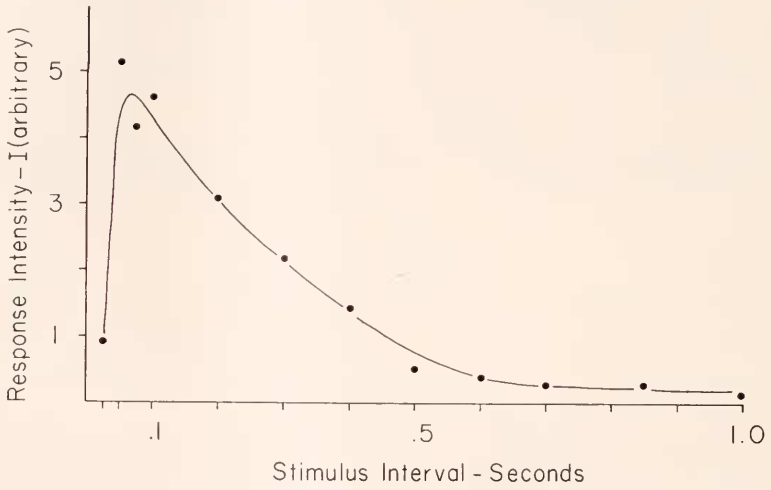


FIGURE 19. Temporal facilitation of electrically induced flashes in the larval firefly. Abscissa as in Figure 18. Stimulus 10 mscs./4 v. Iowa *Photuris* larva, isolated light organ.

15 per second (Fig. 33), *Photinus punctulatus* reaches its limit at about 10/sec. (Fig. 23), the Iowa *P. pyralis* (Fig. 31) and *P. marginellus* at 7/sec., while the Maryland *P. pyralis* barely follows at 4/sec. even with intense stimuli.

Up to the species limit, the ability to respond 1 : 1 to a particular frequency of stimulation increases with either increasing voltage or increasing stimulus duration. The actual frequency limit shows a definite relation to the strength-duration product in that 1 : 1 response can be maintained by a variety of duration-voltage combinations. In *P. marginellus,* for example, the frequency limit of about 7/sec. applies at stimuli ranging from 50 mscs./3 v. to 3 mscs./50 v. However, neither near the limit of 1 : 1 response nor at more moderate frequencies does there appear to be a true reciprocity in the response itself, *i.e.,* in mean flash intensity (Fig. 32).

An effect commonly seen at the frequency response limit is a rather regular alternation in flash intensity (Figs. 28, 30, 33). At stimulation frequencies exceed-

ing the limit of 1 : 1 response, the response differs somewhat depending on species, on stimulus parameters, and on whether or not the lantern is deganglionated, but generally resembles either a more or less typical tetany (Figs. 24, 25) or consists of serial responses to separate stimuli at some exact fraction of the actual stimulation frequency (Figs. 31, 34, 35).

d. *Adaptation and fatigue.* Free-flying fireflies can produce many dozens of consecutive flashes at the species-characteristic frequency without apparent fatigue. Even when flashing excitedly in the laboratory at much higher than normal frequencies, little decrement in either frequency or brightness of spontaneous flashes can be detected (there is about a 20% falloff in mean peak height and a 6% lengthening of interflash interval between the two parts of Figure 1). Repetitive driven flashing may, depending on the conditions of stimulation, either continue practically unchanged for long periods (Fig. 13), go through cycles of varying excitability (Figs. 21, 26, 32), or cease relatively quickly. As an example of one extreme, a female *Photuris* stimulated once per second flashed for more than 70 minutes with only slowly decreasing vigor (over 4000 successive flashes). At the other extreme

TABLE I

Stimulus-response interval at 25° C. Two to six measurements per individual

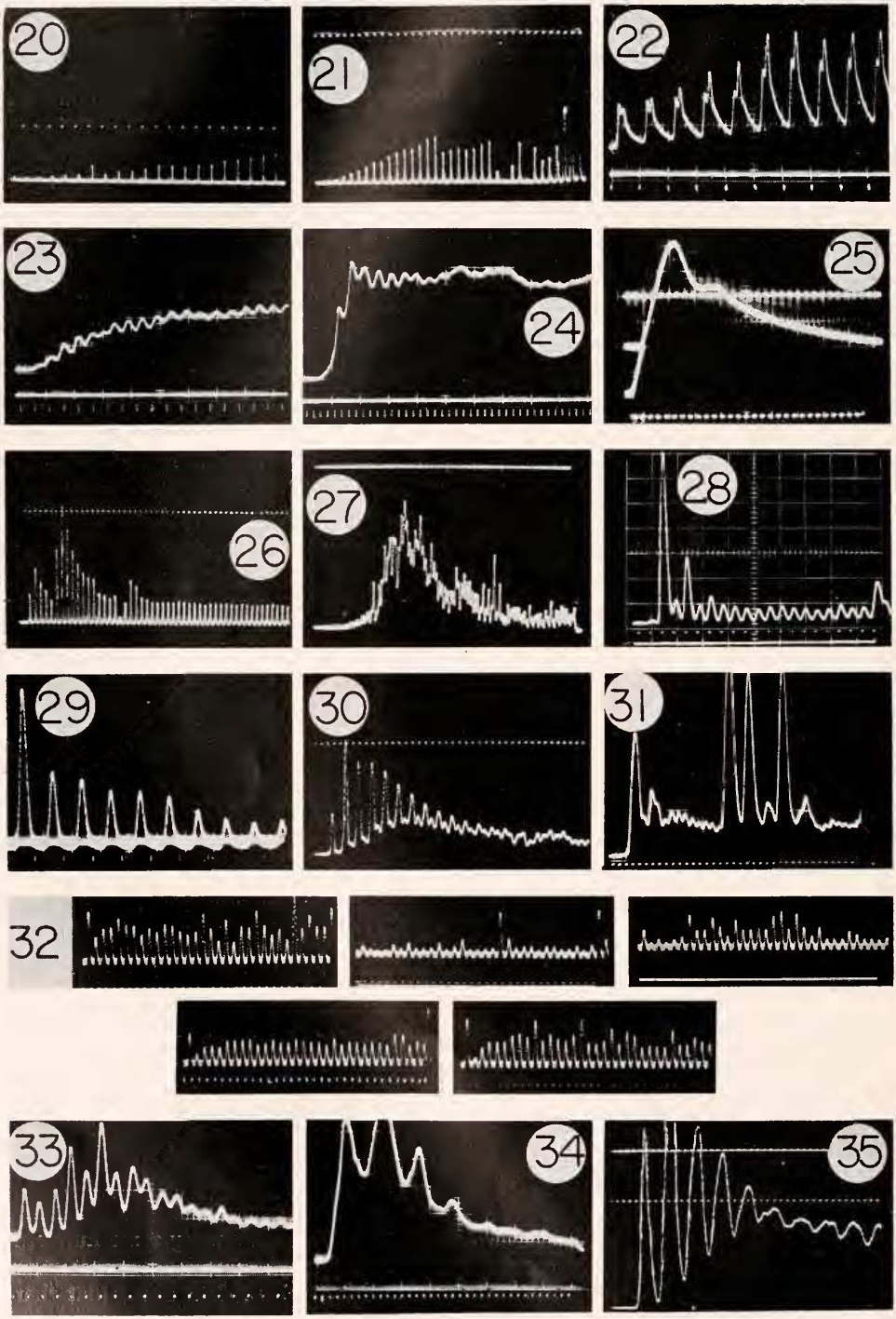
Species	No. individs.	Latency (milliseconds)	
		Average	Range
Photuris	6	86	55–101
Photinus pyralis			
Maryland	4	194	160–206
Iowa	4	166	144–190
Photinus punctulatus, male	10	67	50–85
Photinus punctulatus, female	2		125, 125
Photinus marginellus	2		26, 27
Photinus consanguineus	8	Approx. 100, 175, 250 (3 elements)	
Photuris, larva	1	800	

are records of the sort illustrated in Figures 29, 30 and 38 in which the response decreased greatly or even failed after a few stimuli. Such damping is particularly common when stimulation frequency exceeds the limit of 1 : 1 response (Fig. 34). Rapid degradation in flash intensity in 1 : 1 response series can probably be ascribed to junctional fatigue when shock voltage is high, and to adaptation when stimulus strength is little above threshold.

In addition to frequency effects already described, it was noted that the first response to a train was sometimes conspicuously more intense than the succeeding flashes (Figs. 29, 36, 37, 63, 64).

5. *Latency*

Table I gives a sample of stimulus-response delays from among some hundreds of flashes of good intensity measured in adult fireflies of several species at about 22° C. under comparable conditions of stimulation. These data show that there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may be significant and characteristic latency differences between species, or even possibly between what appear to be regional variants of one species (*P. pyralis*). The considerable latency difference between the sexes of *P. punctulatus* was not found in *Photuris*, and females of the other species are so seldom found that no measurements were obtained.

Considering the probable multiplicity of the excitation pathways and the number of response units in the lantern, response latency within individuals is remarkably constant. Serial responses of comparable intensity given in response to regularly repeated identical stimuli may vary less than 5% of their mean value, particularly in deganglionated lanterns.

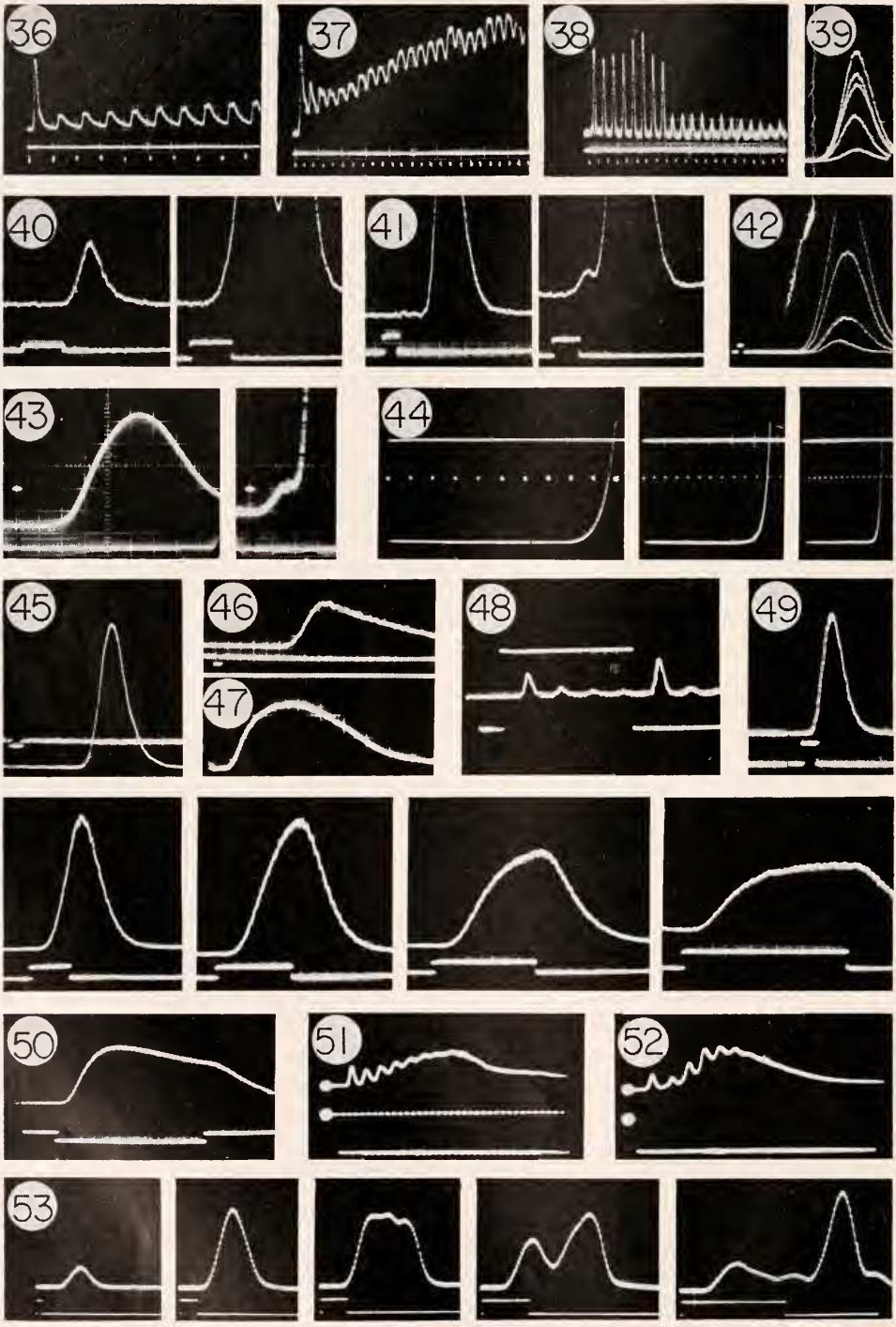
a. *Effects of temperature on latency.* In *Photuris* and in *Photinus pyralis*, our most used species, 8° C. seems to be about the limit to which temperature can be lowered and still permit induced flashing, and even this cannot be accomplished without strong stimulation (see Threshold), change in form (see below), and much prolonged latency (Fig. 54). Above this temperature, latency decreases, at first rapidly. Between 32° C. and 35° C. in *Photuris* there is some indication of an inflection point. In a male studied (not plotted) it was particularly striking. Below this point the temperature-induced changes in latency are fully reversible, but above it permanent damage seems to occur, as indicated by failure of the latency curve to retrace its path as temperature is lowered again.

Since overall latency reflects a complex of conduction and excitation steps, mostly unknown, Q_{10} values are of uncertain worth; however, they lie within the usual range for neuroeffector systems (*ca.* 1.5 at 30° C., and over 2.0 at 20° C.). Temperature characteristics are of even more tenuous significance, but the data appear to yield linear Arrhenius plots up to 30° C.

Though measurements of latency changes of the larval "flash" with temperature are available only for the 14°–26° C. range, differences from the adult pattern are suggested (Fig. 55). First, the trend seems to be linear rather than inflected. Second, the Q_{10} is about 3.0, or about 50% higher than the value for the adult in the corresponding range.

FIGURES 20–31. (20) Woods Hole *Photuris*, male, decapitated, electrodes on prothoracic ganglion. Responses to 10 mscs./15 v. at 2 per second. S = 11 secs. (21) Same as Figure 20 except stimulus frequency 7 per second. S = 5.5 secs. (22) *Photinus punctulatus*, male, isolated lantern. Responses to 2 mscs./10 v., at 2 per second. S = 5 secs. (23) Same as Figure 22 except stimulus frequency 10 per second. S = 2 secs. (24) Same as Figure 22 except stimulus frequency 20 per second. S = 2.1 secs. (25) Iowa *Photinus pyralis*, male, decapitated, electrodes in lantern. Responses to 40 mscs./150 v. at 15 per second. S = 2.5 secs. (26) Woods Hole *Photuris*, male, decapitated, electrodes on prothoracic ganglion. Stimulus 5 mscs./15 v. at 10 per second. S = 5.7 secs. (27) Same as Figure 26 except stimulus frequency 20 per second. S = 5.5 secs. (28) Iowa *Photinus pyratis*, male, decapitated, electrodes in lantern. Stimulus 5 mscs./10 v. at 4 per second. S = 5.5 secs. (29) Iowa *Photuris*, male, decapitated, electrodes in lantern. Stimulus 1 msc./6 v. at 5 per second. S = 2 secs. (30) Woods Hole *Photuris*, male, decapitated, electrodes on prothoracic ganglion. Stimulus 5 mscs./15 v. at 20 per second. S = 2.1 secs. (31) Same as Figure 28 except stimulus frequency 7 per second. S = 5.5 secs.

FIGURES 32–35. (32) *Photinus marginellus*, male, decapitated, electrodes in lantern. Responses to five series of stimuli at 3 per second: 12 mscs./5 v., 20 mscs./3 v., 6 mscs./10 v., 4 mscs./15 v., and 2 mscs./30 v. S = 1 sec. (33) Iowa *Photuris*, male, isolated lantern. Stimulus 2 mscs./20 v. at 10 per second. S = 2 secs. (34) Same as Figure 33 except stimulus frequency 20 per second. S = 2 secs. (35) Woods Hole *Photuris*, male, decapitated, electrodes on prothoracic ganglion. Stimulus 5 mscs./15 v. at 40 per second. S = 2.1 secs.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b. *Effects upon latency of stimulus strength, duration and frequency.* Latency rather frequently appears to decrease progressively with increasing strength of stimulus (Figs. 8, 40). In many instances these effects can be ascribed to the magnitude artifact already mentioned, and this is probably also the explanation of the apparent latency changes seen in responses facilitated in serial stimulation (Figs. 39, 42). Latency may also appear to decrease stepwise due to the becoming visible of an additional response of shorter latency. Such early shoulders on the main flash may appear simply as a consequence of increased amplification of the photomultiplier trace (Fig. 43), or be invoked by increasing stimulus strength (Fig. 40) or duration (Fig. 41).

Striking differences in ostensible latency may occur with trains of subliminal stimuli which exceed the capacity of the lantern for 1:1 response (Fig. 44), although there is ambiguity as to which is the first effective stimulus.

With strong stimulation, some instances of shortened latency are due to the evocation of a separate and qualitatively different sort of early response ("quick flash"—Case and Buck, 1958; 1959b) that is considered in detail in the third paper of this series.

6. *Flash form*

Flash form—the variation in light intensity with time—is subject to so many internal and external influences that few generalizations are possible. We have already alluded to changes in form involved in the transition from single to double flashes (Fig. 14), and similar changes are seen in the evocation of multiple responses by increased stimulus voltage (Fig. 12) and duration (Fig. 53), as well as in spontaneous flashing (Fig. 7). Some factors influence differently the rates of

FIGURES 36–53. (36) *Photinus punctulatus*, male, isolated lantern. Stimulus 2 mscs./10 v. at 2 per second. S = 5 secs. (37) Same as Figure 36 except stimulus frequency 5 per second. S = 5 secs. (38) Iowa *Photuris*, male, isolated lantern. Stimulus 1 msc./5 v. at 5 per second. S = 5.5 secs. (39) Woods Hole *Photuris*, female, decapitated, electrodes immediately anterior and posterior to lantern. Five superimposed responses to 6 mscs./20 v. Temperature 17.9° C. S = 80 mscs. (40) Woods Hole *Photuris*, male, decapitated, electrodes in lantern. Stimulus 60 mscs./4 v. and 60 mscs./15 v. S = 250 mscs. (41) Same as Figure 40 except stimuli 20 mscs./15 v. and 40 mscs./15 v. S = 250 mscs. (42) Iowa *Photuris*, male, isolated lantern. Stimulus 6 mscs./20 v. Temperature 26.7° C. S = 190 mscs. (43) *Photinus consanguineus*, male, isolated lantern with intact ganglia. Two responses to 10 mscs./15 v., the second at 12.5 times the amplification of the first. S = 480, 230 mscs. (44) Maryland *Photinus pyralis*, male, decapitated, electrodes on prothoracic ganglion. Responses to 2 mscs./9 v. at 10, 20 and 40 per second. S = 1100, 650, 440 mscs. (45) Woods Hole *Photuris*, female, decapitated, electrodes in lantern. Stimulus 10 mscs./6 v. Temperature 25.5° C. S = 160 mscs. (46) Same as Figure 45 except stimulus 10 mscs./80 v. Temperature 12° C. S = 600 mscs. (47) Woods Hole *Photuris*, female, decapitated, electrodes in lantern. Stimulus 10 mscs./40 v. Temperature 39.2° C. S = 500 mscs. (48) Woods Hole *Photuris*, male, isolated lantern. Stimulus 400 mscs./8 v. S = 860 mscs. (49) *Photinus consanguineus*, male, isolated lantern. Stimuli 30 mscs./15 v.; 100 mscs./10 v.; 200 mscs./9.5 v.; 300 mscs./9.5 v.; 500 mscs./12 v. S = 250, 306, 340, 410, 390 mscs. (50) *Photinus marginellus*, male, isolated lantern. Stimulus 500 mscs./90 v. S = 850 mscs. (51) Woods Hole *Photuris*, female, decapitated, electrodes in thorax. Stimulus 20 mscs./15 v. at 30 per second. S = 1.4 secs. (52) Same as Figure 51 except stimulus 12 v. D.C. beginning with deflection of lower trace. S = 1.4 secs. (53) *Photinus marginellus*, male, decapitated, electrodes in light organ. Stimuli 15 v. for 20, 80, 100, 200, 500 mscs. S = 800, 650, 700, 950, 1100 mscs.

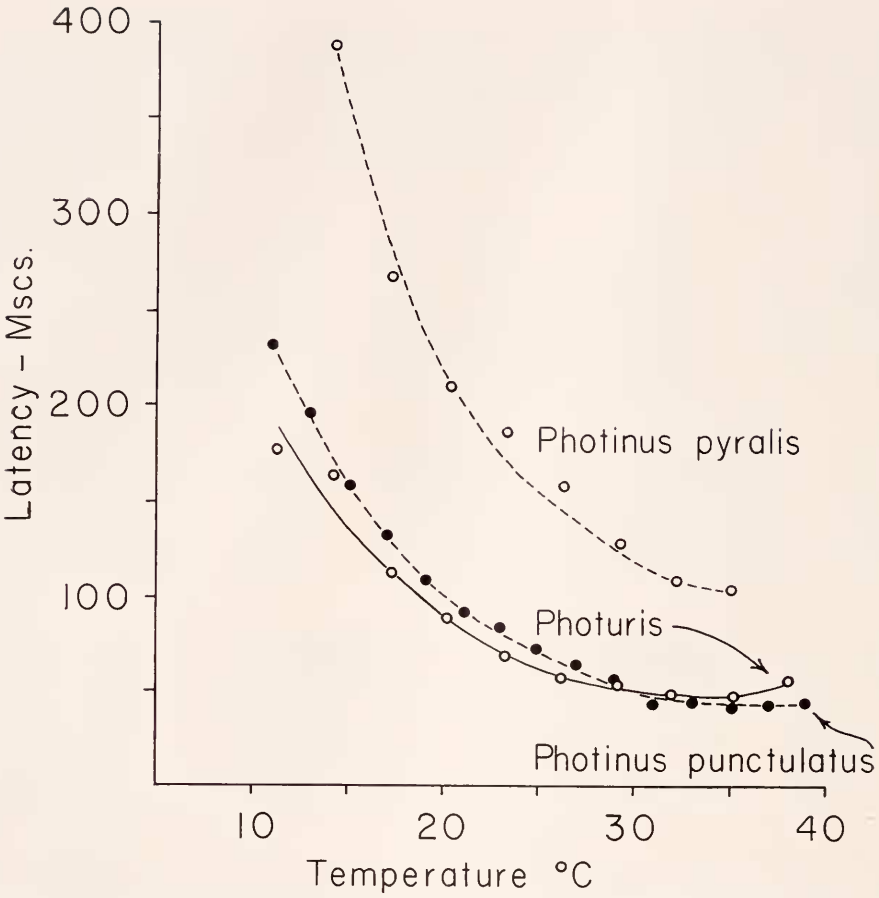


FIGURE 54. Stimulus to response latency in relation to temperature for adults of three species of fireflies. Stimulus varied as function of temperature in order to produce similar magnitude flashes. Points represent means of measurements on 5 male *P. pyralis,* 10 female Woods Hole *Photuris,* 6 male *Photinus punctulatus.* Direct excitation of lantern.

accretion and decay of light intensity. One of the most predictable of these is temperature. Between approximately 15° and 30° C. flash form remains relatively constant (Fig. 56), but temperatures outside this range usually produce a preferential slowing of the decay phase of the flash (Fig. 45 *vs.* Fig. 46). This slowing is usually progressively greater the more extreme the temperature, but in some series there appears to be a relatively sudden form change at around 15° C. The changes toward the ends of the range are difficult to evaluate quantitatively because of the developing steady glow (see below) and the necessarily concomitant changes in stimulus strength. The low temperature skewing seems to be completely reversible, but the slowed decay at high temperature (Fig. 47) persists after return of the animal to normal temperatures, suggesting irreversible damage.

Flash form is of course affected by long or high frequency stimuli that prolong

luminescence. Several such deviations can be induced. A curious effect of lengthening shock duration is a slowed rise demonstrable in flashes of comparable peak intensity given by deganglionated lanterns (Fig. 49). There is also often a perceptible "off effect," of the same latency as the primary rise (Figs. 48, 53). However, it is also clear that the lantern can be stimulated throughout the duration of long stimuli, since a flash of many times normal duration can be produced (Figs. 49, 50). Presumably the same explanation applies to the fact that trains of stimuli above the frequency limit of 1 : 1 response (Fig. 51) may produce the same effect as the continuous passage of current (Fig. 52).

There seem clearly to be elements of both facilitation and of fatigue or adaptation in artificially lengthened flashes. Depending on whether the voltage is low or high, shocks of equal duration may induce either flashes which increase in intensity up to the break point (Fig. 49, fifth frame) or which start high and decline steadily (Fig. 50).

Customarily the peak positions in a homologous series of flashes coincide rather closely (Figs. 39, 57), indicating that the *rate* of rise of light intensity is independent of total light production. However, in some series the peak positions of

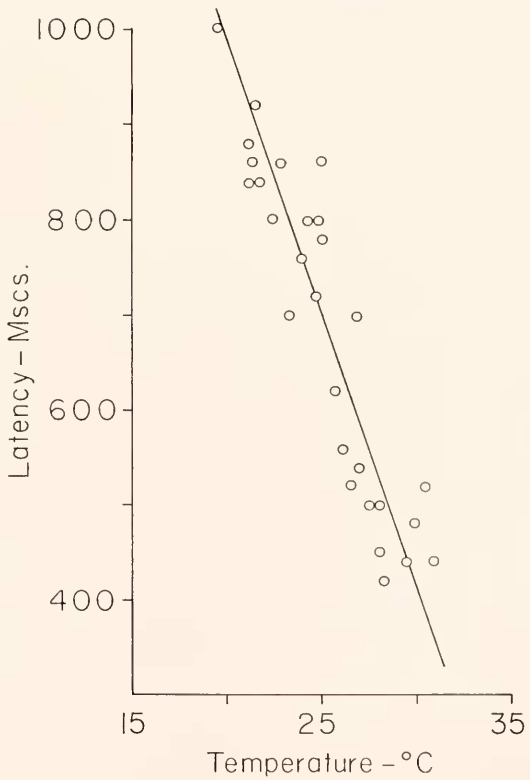


FIGURE 55. Stimulus to response latency for Iowa *Photuris* larvae. Stimulus strength varied with temperature to produce similar magnitude flashes.

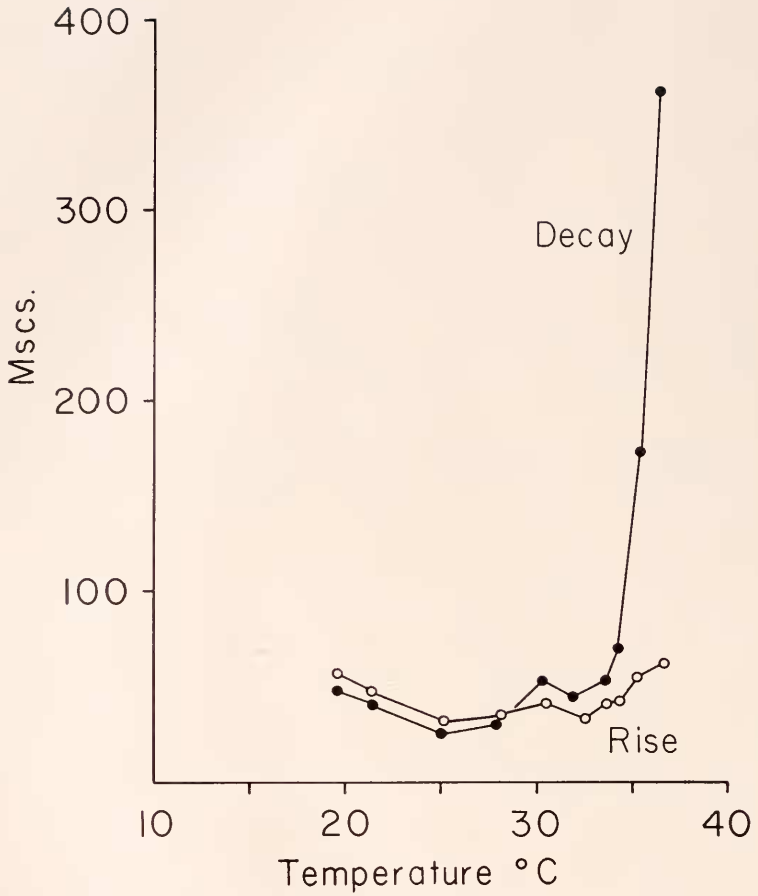


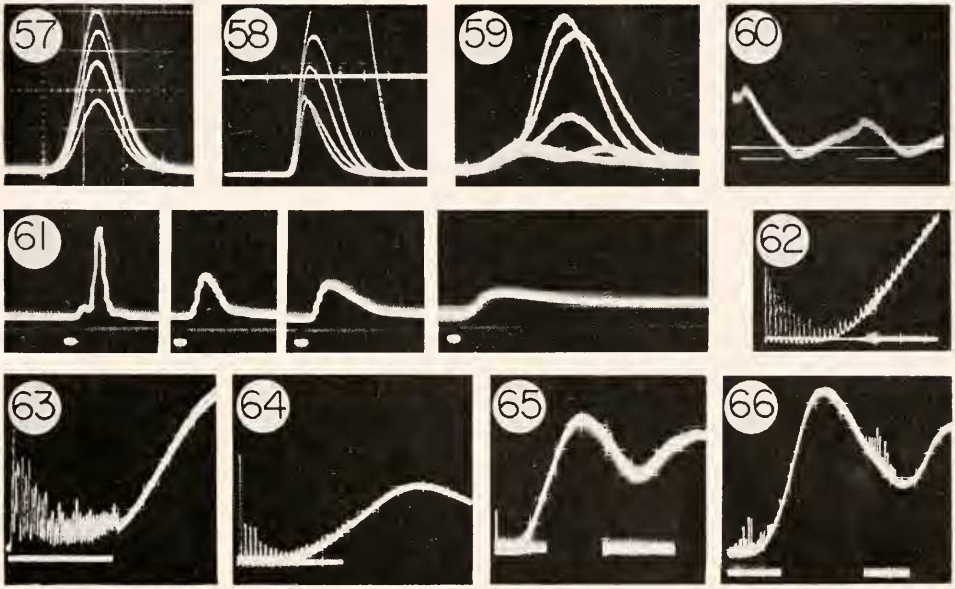
FIGURE 56. Influence of temperature on flash form as indicated by variation in half-rise and half-decay times of flashes induced by electrical excitation of lantern. Stimulus strength varied with temperature in order to produce as nearly equal magnitude flashes as possible. Iowa *Photuris* male.

the low intensity flashes are variable, usually, but not always, in the direction of early peaking (Figs. 58, 59).

Numerous other interesting alterations in flash form of even more obscure causation have been observed. When, for example, a firefly is stimulated every few seconds in decreasing ambient oxygen concentration the decay phase of the flash sometimes tends to be slowed much more than the rise phase as hypoxia deepens (Fig. 61).

7. Glow

In addition to the various types of more or less brief flash, all species of firefly may show moderate to long-continuing steady luminescences which vary widely in



FIGURES 57–66. (57) Woods Hole *Photuris*, female, decapitated, electrodes in prothoracic ganglion. Four superimposed responses to 10 mscs./10 v. S = 100 mscs. (58) Woods Hole *Photuris*, female, intact, with electrodes on prothoracic ganglion. Stimulus 1 msc./7 v. Temperature 22° C. S = 470 mscs. (59) Maryland *Photinus pyralis*, male, decapitated, electrodes in lantern. Five superimposed responses to 5 mscs./48 v. Temperature 13° C. S = 1 sec. (60) Iowa *Photuris*, male, isolated lantern. Response to two trains of 10 mscs./50 v. at 30 per second. S = 45 secs. (61) *Photinus punctulatus*, male, decapitated, electrodes in prothorax. Electrically excited flashes at 0, 18, 34, and 46 secs. after start of exposure to N_2. S = 760, 520, 680, 1220 mscs. (62) Iowa *Photuris*, male, isolated lantern. Stimulus 1 msc./6 v. at 5 per second for duration of sweep. S = 8 secs. Note that in Figures 63–66 stimulation is indicated by unblanked portion of signal trace. (63) Same as Figure 62 except stimulus 1 msc./5 v. at 15 per second. S = 4.2 secs. (64) Same as Figure 62 except stimulus 1 msc./6 v. at 5 per second. S = 9.5 secs. (65) Same as Figure 62 except stimulus frequency 10 per second. S = 9 secs. (66) Same as Figure 62 except stimulus 1 msc./8 v. at 15 per second. S = 9 secs.

causation, intensity, area of lantern affected, and rates of accretion and decay. Though these are not well understood, they belong in any inventory of the photogenic potentialities of the lantern.

Glowing is the normal mode of luminescence of the larva, and occasionally the larval light organs persist through the pupal stage and are functional in the adult (in the eighth sternite) in addition to the normal adult lanterns in segments 6 and 7. The female of *Photuris* rather typically emits a protracted glow from her adult lantern while in flight, and apparently normal specimens of other species are occasionally seen to emit similar glows. It is not possible to distinguish visually between such normal glows and the continuous luminescence that commonly develops in strong repetitive stimulation and which presumably represents flash fusion (Figs. 23–25, 27, 30, 31, 34, 35, 37 etc.).

In instances where the photomultiplier records flashes superimposed on glow (Figs. 62, 64, 66) it is generally found upon microscopic examination that glowing

regions do not flash, and vice versa. The relations of glow and flash are in fact complex and enigmatical. Glowing may sometimes be suppressed (Fig. 63) or reduced (Fig. 60) by high frequency electrical stimulation, thereafter rising spontaneously though temporarily. It may also apparently be enhanced by repetitive stimulation, after a lag (Figs. 65, 66), with or without flashing.

Sustained glowing is also often engendered by non-physiological conditions, such as excess heat or cold, injection of many materials into the hemocoel, toxic vapors, high or low pO_2, death, etc., and it seems probable that there are several types of "glow," perhaps none of which is analogous to the normal glows of larva and adult, or to the transitory glowing associated with vigorous stimulation.

Microscopic examination of the lantern surface contributes relatively little to our understanding of glows. In most instances, as indeed in flashing, the tissue seems to be uniformly alight, and no fine detail can be made out. The impression is often given that the active tissue is deep in the organ, but there is no histological ground for such a distinction (Buck, 1948) nor is it really possible to distinguish between self-luminosity in surface tissue and secondary glowing due to light diffusing through from a deeper source.

Discussion

Different species of firefly have long been known to differ characteristically in color and intensity of light produced and in flash pattern and duration. The present findings give quantitative expression to the differences in flash duration and form, and show that interspecific distinctions extend to such details of excitation as latency and frequency response limit. No correlations are yet apparent between response characteristics and other species differences such as behavior, body size or lantern structure, except in the instance of the larva, where sluggishness of response is associated with a minute and primitively constructed photogenic organ.

From the electrophysiological standpoint the flash response has so many similarities with conventional neuroeffector excitation systems that direct neural control can scarcely longer be doubted. In regard to the specific analogy with muscle (Chang, 1956), the flash is leisurely and its response latency long compared with a striated muscle twitch, but there is rather good qualitative correspondence between the two systems in regard to strength-duration relations, temperature effects, the effects of changing stimulus voltage, duration and frequency, and the courses of fatigue, adaptation and facilitation. Although we are no nearer to having a concrete picture of how nerve and photocyte are associated, the evidence is strong that the association has many of the properties of a conventional neuroeffector junction.

The remarkable species-specific constancy in normal light emission is coupled with an equally remarkable lability to experimental influences, and it is this capacity for varied response that shows that we are very far from being able to define the basic excitatory event. *A priori*, flash form should be of special interest because of the possibility that it might yield kinetic information. However, in view of the facts that the lantern is estimated to contain of the order of 600,000 photocytes (Buck, 1948) which are controlled in localized groups by peripheral nerve twigs (Hanson, 1961), any hope that the flash mirrors directly the time course of either the basic chemiluminescence or the excitation process is illusory. It has in fact been argued (Buck, 1955) that when light from numerous unresolved foci is de-

tected by an integrating device such as eye or photomultiplier tube, symmetrical, skewed or multiple flashes of any desired form can theoretically be produced merely by varying the gross sequence and spread of excitation. Therefore, although it is possible to give plausible explanations of many of the luminescent phenomena here recorded in terms of conventional interplay between excitation and response mechanisms, it seems preferable to defer most such attempts until conductional, junctional and effector processes can be separated clearly, and the experimental preparation can be limited, if not to single units, at least to constant effector populations.

Nevertheless, the flash is not totally devoid of information. The preferential slowing of the decay phase of the flash at low temperatures (Fig. 46) and in hypoxia (Fig. 61) indicates that the rise and extinction processes are qualitatively different, rather than being merely the two phases of a reversible process. The lability and variability of decay also suggest that it reflects some sort of active control process rather than, for example, dieaway of the ultimate chemiluminescence. The slowing effect of hypoxia on decay of luminescence might be similarly interpreted as interference with a metabolic extinction process. It could also reflect direct oxygen-limitation of the chemiluminescence; however, the virtual absence of fatigue in moderate repetitive stimulation indicates that actual exhaustion of some reactant in light production is unlikely ever to become rate-limiting in the normal over-all response.

Even in the mass flash of the whole lantern there are sometimes clear indications of functional heterogeneity. The flashing of *Photinus consanguineus* is especially interesting in this respect since it normally shows three peaks which have constant latencies (Table I) but independently modulated amplitudes (Fig. 7). The fixed sequence of excitation, shown by both intact and decapitated specimens—but not, significantly, by deganglionated lanterns (Fig. 49)—undoubtedly reflects central nervous programming, while the variable intensities of the three peaks suggest effector populations of variable sizes. Similarly, the early shoulders evoked by intensified stimulation (Figs. 40, 41) probably reflect recruitment of additional groups of effector units with higher threshold and shorter latencies.

A second point of interest about the spontaneous flashing of *P. consanguineus* (Fig. 7) is that whereas each of the three peaks varies continually and markedly in intensity, the total light emitted in each of the last four over-all flashes has an extreme range of variation of only 8% of the mean value, and the first flash is only 25% low. This raises the interesting possibility that the effector units are excited in relays so that total light per over-all flash is kept constant without all photocytes having to participate in each flash. In single-flashing fireflies the maintenance of a uniform flash presents no particular problem, assuming uniform excitation, but the regulation apparently practiced by *P. consanguineus* would appear to require some sort of sensory feedback.

In the pair-shock series shown in Figure 14, both stimuli occur in advance of the response (as long as it is single), so the changes in rise slope and peak position seem reasonably explicable on the basis of conventional facilitation. In the stimulus duration series shown in Figure 49, however, the lantern has no way of knowing, at the time its response begins, how much longer the current will flow—and yet the rise slope progressively flattens and the peak shifts to the right as the stimulus lengthens. Broadening of the flash seems scarcely ascribable to developing inhibi-

tion or to a rescheduling of the firing times of the effector units, because total light increases. It seems, therefore, that although direct current flow may stimulate continuously, producing a response analogous to constant-current contracture of muscle (Fig. 49, last frame; Fig. 50), there may also occur interaction of on and off effects of the pulse. When the pulse is short (Fig. 49, first and second frames) the interaction resembles facilitation and increases the flash height, whereas when start and finish of the pulse are widely separated the effect is seen mainly in a much-prolonged decay phase. Lack of the break effect might also explain why the D.C. threshold is higher than that determined with pulses. In some circumstances, possibly when deganglionation has removed the potential of continuous neural re-excitation, the make and break effects of long pulses occur separately (Fig. 48).

In repetitive spontaneous flashing it is of some interest to inquire how successive flashes are interrelated in magnitude and timing. If we assume a neural pacemaker of not absolute regularity it would be expected that, because of persisting facilitation, shorter interflash intervals would be correlated with more intense succeeding flashes and longer interflash intervals with smaller succeeding flashes. An alternative possibility would be that flash intensity is a function of concentration of substrate available for luminescence—in which case big flashes (using more substrate) should tend to be followed by small, and big flashes should tend to be preceded by longer than average interflash intervals (needed for substrate replenishment). An analysis of the 80 flashes and 79 intervals in the series illustrated in Figure 1 revealed that (a) the flashes following short intervals were 5% more intense than average, (b) the flashes following long intervals were 18% smaller than average, (c) the flashes following big flashes were 7% larger than average, and (d) the flashes following small flashes were 11% smaller than average. We construe these findings to indicate that flash intensity reflects degree of neural facilitation rather than substrate concentration.

The phenomenon or phenomena of induced glowing raises questions of great potential interest, but in view of the paucity of definite information it need only be said that (a) the irreversible, homogeneous, unresponsive, oxygen-sensitive, unchanging glows that are induced by various mechanical or chemical injuries probably represent the uncontrolled luminescing of the substrate stored in the photogenic tissue and are different from either the transitory decay tails of some flashes or the reversible glows associated with high frequency stimulation, (b) the sluggish rate of change of glows, even those responsive to stimulation, cautions against considering them neurally-mediated responses in any usual sense, (c) glows that decay either spontaneously or under stimulation, or glows that augment with stimulation, have some counterparts in flashing behavior; but glows that augment in the absence of stimulation (*e.g.,* Fig. 63) seem to pose an especially interesting problem for the future.

The glow of the firefly larva presumably belongs in a separate category since it is the normal mode of lighting and since it is responsive to quite modest stimulation intensities. In view of the long-known absence from the larval lantern of the tracheal end cells or end organs that form such a characteristic feature of the adult lantern, it seems reasonable to regard the tracheal end cells as being somehow associated with the ability to delimit luminescence sharply. It does not follow, however, that glowing in the adult necessarily means end cell inactivation.

Summary

1. Records are presented of normal spontaneous flashes and of flashes induced by a variety of electrical stimuli at a variety of anatomical sites in several species of lampyrid firefly.

2. The flashes of adult firefly lanterns have long response latencies (25 to 250 mscs. at 25° C. in different species) and durations (100 to 1000 mscs.) and can be repeated many hundred times with only slight fatigue. The response itself shows strength-duration relations and frequency responses (summation, treppe, tetany) which are similar to those of more conventional neuroeffector systems. A striking long-lasting neuroeffector facilitation is also evident.

3. Response latency lengthens with falling temperature, Q_{10} values for the 10°–30° range varying from about 2.4 to 1.4. Extreme temperatures slow the decay phase of luminescence preferentially, as does hypoxia.

4. The flashes of most species differ characteristically in time course, response latency and other electrophysiological properties.

5. The responses of the *Photuris* larva are roughly similar to those of the adult, but slower by a factor of about 10.

6. The time course of light intensity change during flashes induced under various conditions is discussed in possible relation to underlying excitation and effector mechanisms. Long lasting glows are also considered in this context.

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